

EXPERIMENT NO: 5

DATE:

ESTIMATION OF DNA

AIM:

To estimate the amount of DNA present in the given unknown solution by diphenylamine method.

PRINCIPLE:

When DNA is treated with diphenylamine under the acidic condition a bluish green colored complex is formed which has an absorption peak at 595nm. This reaction is given by 2 deoxypentose in general. In acidic solution deoxypentose are converted into a highly reactive β hydroxyl leavulinic aldehyde which reacts with diphenylamine gives bluish green colored complex. The colour intensity was measured using a red filter at 595nm.

REAGENT REQUIRED:

1. Stock Standard Solution:

50mg of DNA was dissolved in 50ml of Saline Sodium citrate buffer. Concentration 1mg/ml

2 .Working Standard Solution:

5ml of stock solution solution was diluted to 50ml with distilled water. Concentration 100 μ g/ml

3. Diphenylamine Reagent:

10g of pure diphenylamine was dissolved with 25ml of concentration sulphuric acid which was made up to 1ml with glacial acidic acid the solution must be prepared freshly.

4. Buffered Saline ph 7.4:

0.14N Sodium chloride and 0.02M sodium citrate.

5. Unknown Solution:

The given unknown solution is mad up to 100ml with distilled water.

PROCEDURE:

1. 0.5-2.5ml of working standard solution is pipetted out into 5 test tubes labeled as s1-s5 where concentration ranging from 50-250 μ g.
2. 1ml and 2ml of unknown solution is pipetted out into two test tube u1 and u2.
3. The volume in all test tubes is made up to 3ml with distilled water and 3ml of distilled water alone serve as a blank.
4. 4ml of diphenylamine reagent was added to all the tubes. The tubes were kept in a boiling water bath at 36°C for 20min. The tubes were than cooled and the bluish colour developed is read at 595nm.
5. A standard graph is drawn taking concentration of DNA on x-axis and absorption of y-axis. From the standard graph the amount of DNA present in the unknown solution is calculated.

RESULT:

The amount of DNA present in the given unknown solution is found to be